Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-11, 14 and 37 are pending in the application, with claim 1 being the independent claim.

Based on the above amendments and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

I. Objection to the Specification

In the Office Action at page 2, section 3, the specification was objected to for containing identical sequences with different SEQ ID NOs. By the foregoing amendments, Applicants have amended the specification to identify each sequence by a unique SEQ ID NO. Applicants respectfully request that the objection to the specification be withdrawn.

II. Objection to the Claims

In the Office Action at page 2, section 4, claims 7 and 8 were objected to as claiming identical sequences. By the foregoing amendments, Applicants have amended the claims to identify each sequence by a unique SEQ ID NO. Applicants respectfully request that the objection to the claims be withdrawn.

III. Rejection under 35 U.S.C. § 112, Second Paragraph

In the Office Action at page 2, section 5, claims 5-7, 11, 12 and 14 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point

out and distinctly claim the subject matter which Applicants regard as the invention. By the foregoing amendments, Applicants have cancelled claim 12, thus rendering its rejection moot. Applicants have also amended claims 5-7, 11, and 14 to correct antecedent bases. Therefore, Applicants respectfully request that the outstanding rejection be withdrawn.

IV. Rejection under 35 U.S.C. § 112, First Paragraph

In the Office Action at page 3, section 7, claims 1-14 and 37 were rejected under 35 U.S.C. § 112, first paragraph. According to the Examiner, the specification did not reasonably provide enablement for a compound that does not have biological activity. Applicants respectfully traverse this rejection. However, in the interest of expediting the allowance of the above-captioned application, Applicants have amended claim 1 and cancelled claim 13 in order to address the Examiner's concerns.

The Examiner rejected claims 1-4, 6, 13 and 37 under 35 U.S.C. § 112, first paragraph, for claiming compounds comprising a linker of undefined size and structure that will require undue experimentation. By the foregoing amendments, Applicants have cancelled claim 13, thus rendering its rejection moot. Applicants respectfully traverse this rejection as it may apply to the remaining claims.

In one aspect, the application as currently claimed is related to novel polypeptides comprising active minimized signaling and binding domains of PTH or PTHrP, linked by a linker. The Examiner cited the lack of working examples directed to linkers other than polyglycine as a factor that could lead to undue experimentation. However, Applicants are not required to provide experimental examples of each and every functional derivative that

Co., 927 F.2d 1200, 1237, quoting *In re Angstadt,* 537 F.2d 498, 502, 190 U.S.P.Q. (BNA) 214, 218 (CCPA 1976) ("it is not necessary that a patent applicant test all the embodiments of his invention. . . . what is necessary is that he provide a disclosure sufficient to enable one skilled in the art to carry out the invention commensurate with the scope of his claims."). Applicants have discovered, *inter alia*, that PTH and PTHrP peptides can be minimized, while retaining signaling and binding activity, by linking the signaling domain via a linker to the binding domain. Thus, although one of the features of the invention is the linking of the domains via a linker, the nature of the linker itself is secondary. Nonetheless, Applicants have provided ample guidance as to the type of linkers that are suitable for use with the present invention (see, *e.g.*, page 32 of the specification), and have demonstrated the practical feasibility of several of these linkers.

Moreover, the linkers that were mentioned in the specification are examples of linkers that were well known to the skilled artisan in protein biochemistry and were commercially available or could have been easily synthesized at the time of the invention. Applicants need not supply information that is well known in the art. *See In re Howarth,* 654 F.2d 103, 105-6, 210 U.S.P.Q. 689, 692 (C.C.P.A. 1981); *Genentech Inc. v. Novo Nordisk A/S* , 108 F.3d 1361, 1366, 42 U.S.P.Q.2d 1001, 1005 (Fed. Cir. 1997); and *In re Brebner*, 455 F.2d 1402, 173 U.S.P.Q. 169 (C.C.P.A. 1972). Furthermore, one of ordinary skill in the art is deemed to know not only what is considered well known in the art but also where to search for any needed starting materials. *See In re Howarth*, 654 F.2d 103, 105-6. Therefore, once a person skilled in the art is in possession of Applicants' disclosure, that person will need to do no more than routine optimization experiments to produce compounds encompassed by the scope of the claims.

The Examiner rejected claims 1-5, 13 and 37 under 35 U.S.C. § 112, first paragraph, for claiming a compound in which the B fragment is a binding portion of PTH, but the binding specificity is not defined. By the foregoing amendments, Applicants have cancelled claim 13, thus rendering its rejection moot. Applicants respectfully traverse this rejection as it may apply to the remaining claims.

The binding specificities of PTH and PTHrP were well known to the skilled artisan at the time of the invention, as evidenced by relevant literature published prior to the filing of the above-captioned application (see, *e.g.*, Gardella *et al.*, "Analysis of Parathyroid Hormone's Principal Receptor-Binding Region by Site-Directed Mutagenesis and Analog Design," *Endocrinol.* 132:2024-2030 (1993)). In the interest of expediting the allowance of the above-captioned application, Applicants have amended claim 1 to more clearly point out and claim the subject matter of the invention.

The Examiner rejected claims 1-12, 14 and 37 as being directed to a compound or polypeptide with no limitation regarding its biological activity.By the foregoing amendments, Applicants have cancelled claim 13, thus rendering its rejection moot. Applicants respectfully traverse this rejection as it may apply to the remaining claims. However, in the interest of expediting the allowance of the above-captioned application, Applicants have amended claim 1 and cancelled claim 13 in order to address the Examiner's concerns.

In view of the foregoing amendments and explanations, Applicants respectfully request that the rejection of claims 1-11, 14 and 37 under 35 U.S.C. § 112, first paragraph, be

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

The following paragraph is sought to be substituted for the paragraph starting on page 11, line 4:

Figure 5. Alanine-scan of PTH(1-14). Shown are the bioactivities of 14 different PTH(1-14) derivatives, each having a different amino acid of the native sequence (SEQ ID. NO:73; shown at bottom of figure) replaced by alanine. Peptides were chemically synthesized, purified and tested for ability to stimulate cAMP formation in COS-7 cells expressing the cloned human PTH-1 receptor. Pepdites were tested in duplicate (± s.e.m.) At a dose of 67 μM. As a control, untreated cells, indicated by basal, were measured. The PTH(1-14) containing alanine at position 1 was used as the wild-type reference. Cells were stimulated for 30 minutes at 21 °C. This figure provides information relevant to bioactivities of amino acid residues in the PTH(1-9) peptide used in the invention.

The following paragraph is sought to be substituted for the paragraph starting on page 13, line 12:

Figure 17. Nucleotide sequence (SEQ ID NO:61) and corresponding amino acid sequence (SEQ ID NO:62) of hP1R-Tether-1 (hP1R-Tether(1-9). Made from the human PTH-1 receptor by replacing Ala24 to Arg181 with Ala1 to His9 of PTH. HK-Tether-1: Sequence ID#: E20986A1 (99nts) and its translation. Oligo to construct Tether-1 in hPTH-1 rec (HK). Join Ala-23 of rec to Val-2 of PTH(1-9)--Glyx4, --Glu-182

ctctgctgccccgtgctcagctccgcgtacgcgGtttCCGAAAtCCAGCtGAtGCACggc-

L C C P V L S S A Y A V S E I Q L M H G -

ggaggaggcgaggtgtttgaccgcctgggcatgatctac (SEQ ID NO: 50)

G G G E V F D R L G M I Y (SEQ ID NO: 55).

The following paragraph is sought to be substituted for the paragraph starting on page 14, line 1:

Figure 19. Nucleotide sequence (SEQ ID NO:57) and corresponding amino acid sequence (SEQ ID NO:58) of hP1R-[R11]-Tether(1-11). Made from hTether-1 by inserting Asn10-Arg11 between His9 and first Gly of linker. Fig 19 Sequence ID#: E27309A1. hThr-Arg11:Insert Asn-10 and Arg11 into

HK-Tether-1 *** Adds NSiI site at Met8/His9 (ATGCAt)

CCGAAAtCCAGCtGAtGCAtAAtCGtggcggaggaggcgaggtgtttg (SEQ ID NO: 69)

E I Q L M H N R G G G G E V F D (SEQ ID NO: 70).

The following paragraph is sought to be substituted for the paragraph starting on page 73, line 25:

Depicted in Figure 11 is the chemically synthesized oligonucleotide (oligo) (#E16631A1) that was used to construct the chimeric rat PTH-1 receptor, rTether-1, which contains at its N-terminus residues (1-9) of rat PTH (A-V-S-E-I-Q-L-M-H-) (SEQ ID NO: 74) fused to Glu-182 of the receptor via a tetraglycine linker. The oligo thus encodes the rPTH(1-9) ligand sequence and four Gly residues in its central portion, and rPTH receptor residues as flanking portions. Also shown is the control oligo (E16853A1) that is similar to E16631A1 but in place of rPTH(1-9) there is the amino acid sequence (P-Y-D-V-P-D-Y-A-) (SEQ ID NO: 71) corresponding to the HA epitope tag; this will yield a receptor construct that we described previously (Luck et al., 1999 Mol.Endo , 13; 670-680).

The following paragraph is sought to be substituted for the paragraph starting on page 77, line 9:

PTH Receptor mutagenesis and COS-7 cell expression: The pCDNA-1–based plasmid encoding the intact hPTH-1 receptor (HK-WT in reference (Schipani, E., *et al., Endocrinol. 132*:2157-2165 (1993)) and herein called hP1R-WT) was used for studies in COS-7 cells. The truncated human PTH-1 receptor (hP1R-delNt) (Figure 18) was constructed from the HK-WT plasmid by oligonucleotide-directed mutagenesis (Kunkel, T.A., *Proc. Natl. Acad. Sci. USA 82*:488-492 (1985)). This mutant receptor is deleted for residues 24 to 181 and, assuming that signal peptidase cleavage occurs between Ala^{22} and Tyr^{23} (Nielsen, H., *et al., Protein Engineering 10*:1-6 (1997)), is predicted to have Tyr^{23} as the N-terminal residue joined directly to Glu^{182} located at or near the boundary of the first transmembrane domain. A similarly truncated rat PTH receptor was described by us previously (Luck, M., *et al., Molec. Endocrinol. 13*:670-680 (1999)). The tethered human PTH-1 receptor [hP1R-Tether(1-9)] (hTether-1 in Figure 17) is based on the hP1R-delNT construct, and has PTH(1-9) and a four glycine spacer (AVSEIQLMHGGGG) (SEQ ID NO: 72) inserted between residues 23 and 182. Assuming that signal peptidase cleavage occurs between Ala^{22} and Tyr^{23}, hP1R-Tether(1-9) is predicted to have Tyr^{23} as the N-terminal residue joined directly to Ala^{1} of the ligand. Analogs of hP1R-Tether(1-9) were made in a similar fashion. Transient transfections of COS-7 cells were performed using DEAE-dextran and 200 ng of cesium chloride-purified plasmid DNA per well of a 24-well plate, as described previously (Bergwitz, C., *et al., J. Biol. Chem. 272*:28861-28868 (1997)).

The following paragraph is sought to be substituted for the paragraph starting on page 78, line 16:

The study began with the construction of the targeted tethered ligand/receptor constructs, which utilized a previously reported delNT receptor as a point of departure (Luck, M., *et al., Molec. Endocrinol. 13*:670-680 (1999)). This mutant receptor lacks residues 24 – 181 of the extracellular N-terminal ligand-binding domain, and is predicted to have Tyr^{23} as the N-terminal residue joined directly to Glu^{182} following signal peptidase cleavage. In order to construct a tethered ligand/receptor construct (hTether), the following 13 amino acid sequence was inserted between Tyr^{23} and Glu^{182}: Ala-Val-Ser-Glu-Ile-Gln-Leu-Met-His-$(Gly)_4$ (SEQ ID NO: 72). Thus, after signal peptidase cleavage, it is predicted that hP1R-Tether(1-9) should contain (C-term to N-term) the intracellular C-terminal domain, the seven transmembrane helices (and accompanying loops), a short glycine spacer and $[Tyr^{-1}]$-rPTH(1-9). Other tethered ligand/receptor constructs were made in the same fashion, wherein only the sequence corresponding to rPTH(1-9) was expanded in the C-terminal direction by one or two amino acids as in hP1R-[R(1-11) (Figure 19).

The following paragraph is sought to be substituted for the paragraph starting on page 83, line 11:

Interaction between the N-terminal residues of PTH and the region of the PTH receptor containing the extracellular loops and transmembrane domains is thought to be a critical step in receptor activation. This hypothesis was evaluated by replacing the N-terminal extracellular domain of the hPTH-1 receptor with residues (1-9) of rPTH (AVSEIQLMH) (SEQ ID NO: 74) using a tetraglycine linker between His-9 and Glu-182 at the extracellular end of the first transmembrane domain to yield hP1R-Tether(1-9). Expression of hP1R-Tether(1-9) in COS-7 cells resulted in basal cAMP levels that were 4- to 5-fold higher than those seen in control cells transfected with hP1R-wildtype. Extending the ligand

sequence to position-11 and including the activity-enhancing substitution of Leu-11→Arg yielded hP1R-[R^{11}]Tether-(1-11) which resulted in a 20-fold increase in basal cAMP signaling, which approached the maximum agonist-stimulated response attained by hP1R-wildtype. Alanine-scan of hP1R-[R^{11}]Tether-(1-11) revealed that Val-2, Ile-5 and Met-8 were crucial for autoactivation. Thus, tethered-ligand receptor constructs can be used for analyzing how PTH interacts with its receptor and induces G protein coupling, and should help to constrain models of the overall topological orientation of PTH complexed with its receptor.

In the Claims:

Claims 12 and 13 are sought to be cancelled.

1. (Once amended) A compound of the structure or formula S-$(L)_n$-B wherein:

(a) S is an amino terminal signaling functional domain of PTH;

(b) L is a linker molecule present n times; and

(c) B is a [C-] carboxy terminal [portion] domain of PTH(1-34) or PTHrP(1-34)

and wherein said compound is biologically active.

5. (Once amended) The isolated polypeptide of claim 2 [1], wherein L is selected from the group consisting of Gly_5, Gly_7 and Gly_9.

6. (Once amended) The isolated polypeptide of claim 2 [1], wherein B is selected from the group consisting of PTH(15-31) (Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val) (SEQ ID NO:2), PTH(17-31) (Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val) (SEQ ID NO:63), PTHrP (15-31) (Ile Gln Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile) (SEQ ID NO:8), and PTHrP(17-31) (Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile) (SEQ ID NO:12).

7. (Once amended) The isolated polypeptide of claim 2 [1] selected from the group consisting of PG5: Ala Val Ser Glu Ile Gln Leu Met His Gly Gly Gly Gly Gly Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO:3), PG9: Ala Val Ser Glu Ile Gly Gly Gly Gly Gly Gly Gly Gly Gly Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO:5), PG7: Ala Val Ser Glu Ile Gln Leu Met His Gly Gly Gly Gly Gly Gly Gly Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO:6), PrPG5: Ala Val Ser Glu His Gln Leu Leu His Gly Gly Gly Gly Gly Ile Gln Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile (SEQ ID NO:64), PrPG9: Ala Val Ser Glu His Gly Gly Gly Gly Gly Gly Gly Gly Gly (Ile Gln Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile (SEQ ID NO:65) and PrPG7: Ala Val Ser Glu His Gln Leu Leu His Gly Gly Gly Gly Gly Gly Gly Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu Ile (SEQ ID NO:66) and functional derivatives thereof.

8. (Once amended) The isolated polypeptide of claim 2 selected from the group consisting of PG5: Ala Val Ser Glu Ile Gln Leu Met His Gly Gly Gly Gly Gly Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO:3 [9]), PG9: Ala

Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO:5 [11]), PG7: Ala Val Ser Glu Ile Gln Leu Met His Gly Gly Gly Gly Gly Gly Gly Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO:6 [13]), and functional derivatives thereof.

11. (Once amended) The isolated polypeptide of claim 2, wherein:

(b) S is Ser Val Ser Glu Ile Gln Leu Met His (SEQ ID NO: 44);

(c) L is 5-10 glycine residues; and

(d) B is [as] Leu Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val (SEQ ID NO: 45).

Please substitute the following claim 14 for the pending claim 14:

14. (Once amended) The isolated polypeptide of claim 2, encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16 [nucleic acid (SEQ ID NO:16) sequence].